K3 51. (amended) The alkalophilic Bacillus strain of Claim 50 wherein said strain is Bacillus novo species PB92 or [a derivative thereof said derivative retaining characteristics of the parent strain] the derivative PBT110.

REMARKS

Claims 41 – 53 are pending in this application. Claims 44, 49 and 51 have been amended to more particularly point out and distinctly claim the invention. Support for the addition of the derivative PBT110 is found *inter alia* at page 16 of the specification.

Claims 44, 49 and 51 have been rejected under 35 U.S.C §112, second paragraph; claim 48 has been rejected under 35 U.S.C. §103(a) as being unpatentable over Fahnestock et al. in view of Aunstrup et al., and claims 41, 42, 45 – 47, 50 and 52 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Fahnestock et al. in view of Aunstrup et al., Hastrup et al., and Dean et al. It also appears the Examiner meant to include a rejection of claims 43 and 53 as unpatentable over the combination of cited references. Applicants respectfully request withdrawal of all above-enumerated rejections.

There are no cited prior art rejections concerning dependent claims 44, 49, and 51. Previous correspondence from the PTO (Paper No. 41, page 8, mailed September 4, 1996 and Paper No. 44, page 9, mailed April 1, 1997) indicated that claims directed to 1) a method of obtaining a non-reverting alkalophilic *Bacillus* strain having no detectable wild-type extracellular high alkaline protease (claim 12); 2) a method for obtaining a mutated high alkaline protease (claim 23); and 3) *Bacillus* strains producing a mutant high alkaline protease (claim 14) were free of the cited prior art. This art included the primary reference, Fahnestock et al., cited against the instant claims.

On December 23, 1998, Applicants filed a response under 37 CFR 1.129 wherein all claims directed to detergent compositions were canceled, and new claims 41 – 53 were presented. These new claims recite the methods and *Bacillus* strains, as stated above in Paper Nos. 41 and 44 to be free of the prior art. While the recitation in the instant claims is not exactly the same as the previous pending claims, the recitations are essentially the same. Instant claim 41 corresponds to previous claim 23; instant claim 48 corresponds to previous claim 12; and instant claim 50 corresponds to previous

claim 14. As argued below, Applicants maintain the instant claims are patentable over the prior cited art.

Regarding the rejection under 35 U.S.C §112, second paragraph, the Examiner states,

> "The meaning of the phrase "or a derivative thereof said derivative retaining characteristics of the parent strain" is uncertain because the specification nor these claims recite the characteristics of the parent strain."

To expedite the prosecution of this application, which has been pending for over 10 years, Applicants have cancelled the phrase "or derivative thereof said derivative retaining the characteristics of the parent strain". While Applicants contend the phrase is not uncertain and would be clear to one of ordinary skill in the art even if not explicitly defined in the specification, the claims incorporating the "derivative" language are dependent claims and the broader generic claims (claims 41, 48 and 50) cover said derivatives.

Applicants stress to establish a prima facie case of obviousness three basic criteria must be met. First, some suggestion or motivation must be found, either in the references themselves or in knowledge generally available to one of ordinary skill in the art, to modify the references or to combine the teachings of the references. Second, reasonable expectation of success must be found in the cited references, and third, the prior art references must teach or suggest all the claim limitations. Furthermore, the teaching or suggestion to make the claimed invention and reasonable expectation of success must both be found in the cited prior art and not based on Applicants' disclosure (In re Vaeck 20 USPQ2D 1438, Fed. Cir. 1991). Applicants contend the cited references do not fulfill these requirements.

Claim 48 has been rejected under 35 U.S.C. §103(a) as being unpatentable over Fahnestock et al. in view of Aunstrup et al. As stated by the Examiner Fahnestock et al. do not teach the method of claim 48. Fahnestock et al disclose methods of obtaining non-alkalophilic *Bacillus* strains which contain an indigenous extracellular non-alkalophilic protease. *Bacillus subtilis* is a non-alkalophilic strain that contains non-alkalophilic subtilisin. The *Bacillus* strains are manipulated to produce very low levels of extracellular protease. In contrast, Applicants' claimed method of obtaining alkalophilic *Bacillus* strains to produce a non-reverting strain having reduced levels of extracellular

high alkaline protease is by a process different than the one disclosed in Fahnestock et al. Not only is the method different but also the final product is different and unobvious. The final product is a non-reverting alkalophilic *Bacillus* strain having a reduced level of extracellular high alkaline protease.

As disclosed in Fahnestock et al., the expression of the indigenous protease was inactivated by two methods. The first method included the construction in vitro of an insertional inactivation mutation in the subtilisin structural gene (apr). The inactivating insert, an antibiotic marker (cat) was inserted into the apr gene. *Bacillus subtilis* hosts were transformed with the construct contained on a plasmid vector. Transformants resulted in very low levels of extracellular proteases. In the second method, a partial deletion of the apr gene was made. Instead of the apr::cat construct, the plasmid vector carried a DNA sequence containing a deletion extending from a H*pa*I site close to the sequence encoding the N terminus of the prepropsubtilisn to an H*pa*I site beyond the end of the apr sequence. Significantly, the plasmid still carried about 1 kbp of chromosomal DNA on either site of the deletion. These sequences would preclude Fahnestock et al. from obtaining a non-reverting *Bacillus* strain. The method according to claim 48 includes transforming an alkalophilic *Bacillus* strain comprising a wild-type high alkaline protease gene with a cloning vector comprising DNA encoding a replication function and 5' and 3' flanking non-coding regions of the high alkaline protease gene, but not the coding region of the high alkaline protease gene wherein a sufficient amount of the 5' and 3' flanking non-coding regions is present to provide for homologous recombination to obtain a non-reverting alkalophilic strain having a reduced level of extracellular high alkaline protease.

While the Examiner states it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of Fahnestock et al., the suggestion or motivation to modify the teaching is not found in the secondary reference.

The secondary reference, Aunstrup et al., is concerned with improving the laundering process by finding proteases produced by various alkalophilic *Bacillus* strains which are better suited for said process than Subtilisin Carlsberg. Aunstrup et al. isolated alkalophilic *Bacillus* strains from soil samples. The proteases from some of these alkalophilic strains were compared to Subtilisin Carlsburg. As shown in Figure 2 of the reference, strain of *B. subtilis* and *B. licheniformis* had maximum growth at about pH 6.2 to 7.2 and about pH 5.8 to 7.2, respectively. In contrast none of the alkalophilic

bacteria could grow at a pH below 6.3 and all had their optimum pH for growth above 8.0.

There is no suggestion provided by the two cited references either alone or in combination that a method of obtaining non-reverting alkalophilic *Bacillus* strains could be obtained with a reduced level of extracellular high alkaline protease. There is no mention in Fahnestock et al. concerning alkalophilic *Bacillus* nor is there any mention of high-alkaline proteases. Furthermore there is no disclosure concerning non-reverting strains transformed according to the present method. The fact that the Aunstrup et al. reference may teach alkalophilic *Bacillus* strains containing high alkaline proteases does not make obvious the claimed method of obtaining non-reverting *Bacillus* strains having a reduced level of extracellular high alkaline protease. The combination of references does not provide any expectation of success.

Claims 41, 42, 45 – 47, 50 and 52 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Fahnestock et al., in view of Aunstrup et al., Hastrup et al., and Dean et al. The discussion above with regard to Fahnestock et al. and Aunstrup et al. applies to the rejection herein of independent claims 41 and 50. However, Applicants contend the cited references are even less germane to these independent claims. Claim 41 is directed to the production of a mutant high alkaline protease comprising obtaining a non-reverting alkalophilic host which is incapable of producing a wild-type high alkaline protease but comprises an integration cassette including a gene encoding the mutant high alkaline protease, and claim 50 is directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease with no detectable wild-type extracellular high alkaline protease. While Fahnestock et al. also teach the heterologous protein, Staphylococcal protein A can be produced in a transformed bacteria wherein the expression of the indigenous protease is inactivated, the reference is devoid *inter alia* of any mention or teaching of a 1) non-reverting alkalophilic *Bacillus* host, 2) which is incapable of producing wild-type high alkaline protease, and 3) which expresses a mutant high alkaline protease. Aunstrup et al merely teaches various alkalophilic *Bacillus* strains.

The Examiner has further cited Hastrup et al. and Dean et al. to support the allegation of obviousness particularly with respect to claims 43 and 53. These claims are directed to asporogenic alkalophilic *Bacillus* strains. Hastrup et al., is cited for teaching that secretion of protease in *Bacillus* is linked to the bacterial growth cycle with

the greatest expression of protease during the stationary phase when sporulation occurs. The Examiner argues inherently production of proteases during sporulation would reduce the levels of recombinant heterologous proteins expressed in *Bacillus* since these proteases would degrade proteins. Dean et al. is cited for teaching methods for creating an asporogenic *Bacillus* sp. Applicants contend independent claims 41 and 50 are not made obvious by the Fahnestock et al. or the Aunstrup et al. reference and the fact that dependent claims 43 and 53 further include limitations of the host such that the host is an asporogenic alkalophilic *Bacillus* strain is not made obvious by a reference which may teach how to create asporogenic *Bacillus* strains or by a reference which teaches that protease production is greatest during sporulation.

In view of the above, Applicants respectfully request that all rejections be withdrawn and that claims 41 – 53 be allowed.

Respectfully submitted,

Date: December 15, 2000

Lynn Marcus-Wyner
Registration No. 34,869
Attorney for Applicants

Genencor International, Inc.
925 Page Mill Road
Palo Alto, CA 94304
Tel: 650-846-7620
Fax: 650-845-6504